Gastrointestinal activity of saponins from soy and tomato

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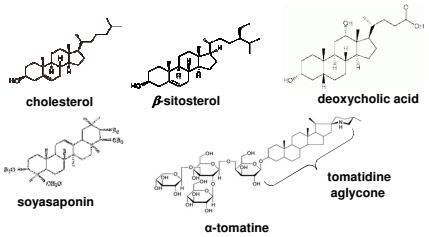
Introduction



Saponins are a diverse family of amphiphilic compounds that consists of triterpene or steroidal nuclei covalently linked to mono- or oligo-saccharides and are found in a variety of edible plants such as soya, tomato, chickpea and fenugreek.[1] Saponins are structurally similar to phytosterols (e.g., β-sitosterol). The hypocholesterolemic activity of phytosterols and phytostanols has resulted in their increased incorporation into food products targeted to decrease plasma cholesterol levels.[5] Limited data from in vitro and animal studies have suggested that soyasaponins also possess hypocholesterolemic activity.

Because saponins are poorly absorbed, adverse effects on the absorption of cholesterol and its bile salt derivatives are likely. The planar steroid ring nucleus of saponins likely interacts with the planar rings of cholesterol and bile salts to impede their respective absorption and re-absorption. This effect results in decreasing plasma and tissue concentrations of cholesterol.[1,2,3] The specific aim of our studies was to determine the effect of saponins isolated from several plant foods on the micellarization of cholesterol during simulated gastric and small intestinal digestion and on the uptake of cholesterol by Caco-2 cells.

Soyasaponins are structurally similar to bile acids, which have been shown to reduce the invasion of *Salmonella enterica* into epithelial cells.[7] The specific aim of these pilot studies was to determine the effect of soyasaponins on the adherence and invasion of *Salmonella Typhimurium* into Caco-2 cells. Thus, saponins may contribute to the health-promoting properties of foods such as soya and tomato.

Abstract

Saponins, a structurally diverse family of secondary plant metabolites, possess anti-carcinogenic, hypolipemic, hypocholesterolemic, and immune-enhancing activities. We have initiated studies comparing the effects of saponins extracted from several sources soy and a commercial mixture of phytosterols (positive control) on GI metabolism and transport of cholesterol and on interactions between gut microbes and intestinal epithelial cells. Saponins from tomato reduced the incorporation of cholesterol into synthetic micelles by 60%, whereas other test compounds (saponins from soya, chickpea and fenugreek and phytosterols) were without effect. During simulated gastric and small intestinal digestion, saponins (90 μM) from tomato and mixed phytosterols significantly inhibited micellarization of cholesterol (14 μM) from a food matrix. Saponins from soy and tomato, as well as phytosterols, also significantly impaired the micellar transfer of cholesterol into differentiated cultures of human intestinal Caco-2 cells. Additionally, soyasaponins decreased adherence and invasion of Caco cells by *Salmonella enterica*. These preliminary observations suggest that saponins from crops important to Ohio's economy may contribute to cardiovascular and gastrointestinal health.

Materials and Methods

Preparation of synthetic micelles. Synthetic micelles were prepared to examine the impact of test compounds on micellarization of cholesterol independent of a food matrix. Stock solutions of phytosterols, soyasaponin, sapogenol, α-tomatine, tomatidine, chickpea, and fenugreek were prepared in dimethylsulphoxide (DMSO; final concentration of DMSO < 0.01%). Aliquots of chloroform containing monoolein, lysophosphatidylcholine, phosphatidylcholine, α-tocopherol, and cholesterol were individually added to 25 mL glass vials before transfer of the solutions of saponins. After addition of radiolabeled cholesterol (5 nCi ^{14}C/mL) to each vial, samples were dried under N_2 gas. Dulbecco's Modified Enriched Medium (DMEM) containing bile salts (glycocholic acid, taurocholic acid, taurodeoxycholic acid), sodium oleate, and glycerol was prepared. Aliquots (20 mL) were transferred to each vial containing the film of lipids and saponins. Vials were sonicated for 30 minutes to form micelles and then filtered (0.22 μM filters). ^{14}C-cholesterol was quantified in filtered and non-filtered aliquots of the samples to determine the extent of micellarization of cholesterol. Final ratio of cholesterol to each test compound was 1:6.5.

Simulated digestion and micellarization of cholesterol in vitro. *In vitro* digestion was conducted using yogurt as the food matrix as described elsewhere.[4] Test compounds included soyasaponin I, soyasapogenol B (aglycone), α-tomatine, and tomatidine (aglycone). The impact of equimolar concentrations (90-93 μM) of test compounds added to the matrix on the micellarization of cholesterol (14 μM with 25 nCi ^{14}C-cholesterol in 100 μL sunflower oil/25 mL reaction) during small intestinal phase of digestion was compared to micellarization during digestion of the matrix containing cholesterol alone. Commercial phytosterol (containing 14 μM cholesterol) served as a positive control as it is known to inhibit micellarization of cholesterol.[5] The ratio of cholesterol to saponins/phytosterol in all test samples was approximately 1:6.5. After digestion, an aliquot of digesta was centrifuged to obtain the aqueous fraction. ^{4}C-cholesterol was quantified in digesta and filtered aqueous fractions of the samples via scintillation counting to determine the extent of micellarization of cholesterol.

Cytotoxicity. Differentiated cultures of Caco-2 cells (HTB37) were exposed to a range of concentrations of soyasaponin, soyasapogenol, α-tomatine, tomatidine, chickpea, and diosgenin for 24 h. Cytotoxicity was assessed by morphological appearance as compared to control cultures. Relative cell number was estimated by Sulforhodamine B (SRB) assay.[6]

Cholesterol uptake by Caco-2 cells. Caco-2 cells (HTB 37; passage 38 at 14 d post-confluency) were incubated in DMEM containing cholesterol and test compounds (at molar ratio of 1:6.5) and incubated in 95% air: 5% CO_2 for 4 h. Monolayers were washed once with ice cold PBS containing albumin (2 g/L) and once with cold PBS alone. Lysis buffer (0.5 mL) was added to each well and shaken on a gyratory shaker for 10 minutes until the monolayer lifted. The cell material was sonicated for 10 seconds and analyzed *via* scintillation counting to determine cholesterol uptake.

Adherence and Invasion of Salmonella Typhimurium to Caco-2 cells. Overnight cultures were grown in LB broth and adjusted to OD = ~ 1. Cultures were back diluted (1:100) in LB medium, 0.1% porcine bile, or 0.1% soyasaponin and grown to mid-log phase (OD ~0.50) Caco-2 cell monolayers were washed 3 times with sterile PBS, and medium (300 μL) containing bacteria was added to cell monolayers and incubated at 37°C for 1 h. Bacteria was added to one dish without Caco-2 cells to determine growth during the incubation period. Following incubation, 1 mL of medium containing gentamicin (216 μmol/L) was added to one set of wells and incubated at 37°C for 2 h to kill extracellular bacteria. Cells were lysed and aliquots plated to determine number of viable bacteria.

Statistical analysis. Data are expressed as means ± SEM and were analyzed by one-way ANOVA and Tukey's Post Hoc pair wise comparison using Minitab for Windows (Minitab v15.1; State College, PA). Differences of p<0.05 were considered significant.

Summary

Cytotoxicity

•α-Tomatine was highly toxic to Caco-2 cells in comparison to other saponins. The greater toxicity corresponds with greater apparent affinity of α-tomatine for cholesterol as observed for the robust inhibitory effect on micellarization during digestion.

Cholesterol Micellarization

Synthetic Micelles

•α-Tomatine significantly inhibited the incorporation of cholesterol into synthetic micelles under the defined conditions, whereas the other saponins were without effect.

Simulated Digestion

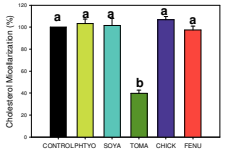
• Phytosterol mixture inhibited cholesterol micellarization ~ 21%, which is similar to inhibition observed in previous studies.[5]

• α-Tomatine was the most potent of the tested saponins for inhibition of cholesterol micellarization (~87%).

Results

Impact of Saponins on Cholesterol Micellarization using Synthetic Micelles

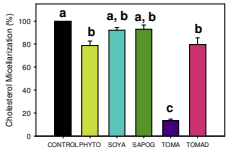
α-Tomatine is potent inhibitor of incorporation of cholesterol into synthetic micelles.*



*Data are means ± SEM (n=3-5) with significant differences (p<0.05) denoted by different superscripts

Impact of Saponins on Cholesterol Micellarization during Simulated Digestion

α-Tomatine is a potent inhibitor of cholesterol micellarization.*



*Data mean ± SEM of n=≥6 with significant differences (p<0.05) denoted by different superscripts.

Cytotoxicity of saponins for Caco-2 cells is proportional to their ability to inhibit micellarization of cholesterol

Compound	Concentration	Impact after 24 h
soyasaponin	≤ 185 μM	No cytotoxicity
sapogenol	≤ 270 μM	No cytotoxicity
α-tomatine	> 25 μM	Cytotoxic
tomatidine	≥ 35 μM	Cytotoxic
chickpea	≤ 200 μM	No cytotoxicity
diosgenin	≤ 200 μM	No cytotoxicity

•Differences in micellarization of cholesterol during the formation of synthetic vs. natural micelles are likely due to greater complexity associated with the digestion of the food matrix.

Cholesterol Uptake

•Soyasaponin, sapogenol, α-tomatine, and tomatidine exhibited similar, although limited, inhibitory activity of cholesterol uptake.

Adherence and Invasion of *Salmonella Typhimurium* into Caco cells

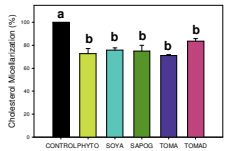
• Adherence of *Salmonella Typhimurium* exposed to soyasaponin to Caco-2 cells was approximately 50% of the control.

•Invasion of *Salmonella Typhimurium* exposed to soyasaponin into Caco-2 cells was approximately 36% of the control.

•Additional studies are planned to confirm pilot results and further define the relationship between soyasaponin and *Salmonella*.

Impact on Cholesterol Uptake

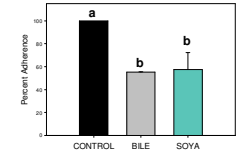
Saponins from soy and tomato inhibit cholesterol uptake by Caco-2 cells.*



*Data are means ± SEM (n=5-6) with significant differences (p<0.05) denoted by different superscripts above the error bars

Adherence of *Salmonella Typhimurium* to Caco-2 Cells

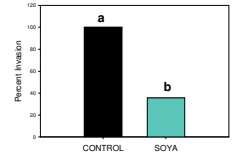
Saponins from soy reduce adherence of *Salmonella Typhimurium* to Caco-2 cells. *



*Data are means ± SEM (n=3) with significant differences (p<0.05) denoted by different superscripts.

Invasion of *Salmonella Typhimurium* into Caco-2 Cells

Saponins from soy reduce invasion of *Salmonella Typhimurium* into Caco-2 cells *



*Data are means ± SEM (n=3) with significant differences (p<0.05) denoted by different superscripts .

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Acknowledgements

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